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博 士 学 位 论 文

典型海洋环境中浮游细菌多样性及环境适应机制的研究

Microbial diversity and Environmental Adaptation Mechanisms in Typical Marine Environments

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典型海洋环境中浮游细菌多样性及环境适应机制的研究

摘　要

海洋浮游细菌是海洋生态系统中丰度最高的微型生物类群，具有极为丰富的遗传多样性和生理代谢多样性，从而具有极为重要的生态地位和功能。本论文阐明了一些典型的海洋浮游细菌类群在中国的典型海域及全球主要大洋中的多样性分布特征，并探讨了它们的环境适应机制以及和环境协同进化的关系。主要研究对象为受关注较少同时对海洋碳循环和光利用有独特贡献的几类浮游细菌类群，包括擅长降解大分子颗粒有机物的嗜纤维菌—黄杆菌类群（*Cytophaga-Flavobacteria*，CF）、生理潜能和生态功能目前仍知之甚少的浮游古菌、具有固定 CO_2 能力的浮游变形细菌、能兼性利用光营混合营养的好氧不产氧光合异养细菌（AAPB）和有色异养细菌（PHB）等。

首先，我们选取了东海的长江口为中国边缘海中河口到陆架的典型过渡区域，系统地调查了 CF 类群、浮游古菌和固碳变形细菌在该区域的多样性分布模式。CF 类群是海洋中最为丰富的浮游细菌类群之一。我们通过使用一对新设计的 CF 特异的 16S rRNA 基因引物构建得到长江口与东海两个海区的克隆文库，揭示了 CF 在这两个区域丰富的多样性以及截然不同的多样性分布模式。70 条 CF 的 16S rRNA 基因序列可分为 26 个亚群，包括 7 个东海特有的亚群、17 个长江口特有的亚群以及 2 个共有的亚群。东海 CF 种的多样性较高，而长江口 CF 亚群的多样性较高。在亚群水平上，长江口和东海都是广谱的亚群占优势地位。来源于土壤、近岸和淡水的亚群仅在长江口有分布，这和该站位的地理位置和环境特征是一致的。CF 种对水文条件完全不同的两种生境的适应以及本土 CF 群落的发展和演替造成了长江口与东海 CF 群落结构间的显著差异。

通过构建长江口古菌 16S rRNA 基因的 2 个克隆文库，我们也揭示出浮游古菌这类在海洋生态系统中广泛分布的类群同样也存在于中国的典型海域。对所得到的 21 个 RFLP 带型测序的结果显示了长江口区域存在着两个古菌类群，即属于 *Crenarchaeota* 的海洋类群 I（MG I）和属于 *Euryarchaeota* 的海洋类群 II（MG II）。MG I 的克隆子在 2 个文库中都是优势类群。我们得到的大部分序列与未培养的海洋古菌近缘，其中有 2 条序列与新鉴定的海洋硝化古菌 *Nitrosopumilus maritimus*

有 98%的同源性，提示古菌在河口生态系统中可能有着重要的生态作用。

具有固碳潜力的变形细菌也是一类具有重要生态意义但受关注较少的海洋浮游细菌类群。我们首次设计得到专门针对变形细菌 1，5-二磷酸核酮糖羧化酶/加氧酶（RubisCO）大亚基（*rbcL*）基因 I 型和 II 型的两套引物，并用于东海近岸和外海两个站位样品的扩增。结果表明 I 型的多样性在高盐低生产力的离岸站位较高，而 II 型的多样性在低盐高生产力的近岸站位较高。部分序列与数据库中已知的 *rbcL* 基因序列相似性很低（60～78%）。揭示出在东海水域分布着多样的具有固碳能力的浮游变形细菌，这也提示我们需要进一步关注它们在海洋碳循环中的贡献。

然后，我们以一类典型的功能海洋细菌类群 AAPB 为代表，系统地分析了它们在全球主要大洋中的多样性分布模式，并探讨了它们和环境的协同进化关系。来自包括太平洋、大西洋和印度洋以及中国边缘海的表层 AAPB 指示基因——光反应中心复合体小亚基基因（*pufM*）的多样性数据表明，AAPB 在全球海洋中具有丰富的多样性，而且随着叶绿素 *a* 浓度的升高而呈降低的趋势。这一发现说明从寡营养到富营养海区，AAPB 的多样性和丰度的分布趋势正好相反。

在垂直梯度上，通过对中心太平洋、大西洋和印度洋三个站位的表层和弱光层上层的浮游细菌样品建立 6 个 *pufM* 基因克隆文库，我们进一步证实了贫营养大洋 AAPB 种群极高的多样性。同时，首次从真光层以下的弱光区域（200 m）扩增得到大量的 *pufM* 序列（共 136 条序列，划分为 37 个 OTU）。系统发育分析的结果表明真光层以下的 AAPB 种群也是多样化的，覆盖了所有在表层海水中发现的亚群，但多样性、GC 和 GC_3 含量比表层站位稍低，同时优势度更高。这些结果支持了这个假说——AAPB 能利用弱光层的弱光来获得能量，从而可以分布到真光层以下的水层中。

为进一步探讨不产氧光合细菌与环境协同进化的关系，我们从公共数据库收集了 89 个含有 *pufM* 序列的不产氧光合细菌及其来源环境相关的信息。系统发育分析表明 21 个 *pufM* 系统发育亚群中有 11 个发生过水平基因转移（HGT）。HGT 不仅发生在同一个纲的物种之间，而且在不同门或亚门的种之间也有发生。不产氧光合细菌的来源环境特征与其系统发育关系密切相关，所有来自有氧栖息地的种和来自缺氧栖息地的种分别聚类在不同的进化枝上。古老的不产氧光合细菌种之间的基因水平转移以及随后它们对各自生态位长期的适应性进化可能导致了不

产氧光合细菌与环境间协同进化的现象。

其次，我们在上述基于不可培养的分子生物学方法研究浮游细菌多样性的基础上，进一步通过培养的方法分析了含色素的有色异养细菌（PHB）这类有重要生态意义的海洋浮游细菌类群的多样性、丰度和光吸收特性。在中国海的近岸、大陆架以及邻近太平洋进行的纯系分离和初步鉴定的研究结果表明，PHB 的丰度和占总可培养细菌数的比例从近岸到外海逐渐降低，在长江口的值最高，分别为 9.9×10^3 个细胞/毫升和 39.6%。在垂直梯度上，PHB 仅分布于真光层，丰度和比重的最高值均出现在表层。对分离得到的 247 株有色细菌进行 RFLP 筛选和 16S rRNA 基因测序的结果表明，它们覆盖了 6 个细菌类群：*α*-变形细菌、*γ*-变形细菌、*Actinobacteria*、*Bacilli*、*Flavobacteria* 和 *Sphingobacteria*，囊括了 25 个属。不同海区的优势类群和种属分布各有差异；同时，不同种属的菌株颜色也各异，包括金黄色、黄色、红色、粉红和橘色等。细胞色素吸收光的波长介于 450～550 nm 之间。这些结果表明 PHB 广泛分布于海洋环境中，并有很高的多样性，它们在海洋生态系统中对光的吸收和利用有着独特而重要的作用。

最后，对海洋微型生物多样性研究中的一些关键性的方法学问题进行了探讨，并建立了两种新的分析方法或标准，包括：通过对数据库中不产氧光合细菌纯系来源的 *pufM* 序列进行以距离为基础的聚类，并将聚类关系与序列来源物种的分类地位相对应，建立了一套种、属、门（或亚门）上的 *pufM* 序列距离标准，分别为 0.06、0.15 和 0.48；通过结合脉冲场凝胶电泳（PFGE）和 PCR 扩增的技术，建立了一种新的准确估算细菌基因组中 16S rRNA 基因拷贝数的方法。

关键词：海洋浮游细菌；*Cytophaga-Flavobacteria*；浮游古菌；固碳变形细菌；好氧不产氧光合异养细菌（AAPB）；有色异养细菌；16S rRNA 基因；*pufM* 基因；*rbcL* 基因；系统发育分析；遗传多样性；中国海；大洋；弱光层；环境适应机制；协同进化；PFGE

Microbial diversity and Environmental Adaptation Mechanisms in Typical Marine Environments

Abstract

Planktonic bacteria are the most abundant microbes in marine ecosystems. They have extremely high genetic and metabolic diversity and thus play a significant role in marine biogeochemical cycles. Here we attempted to shed new light on the diversity, environmental adaptation mechanisms, and co-evolution with environments of some typical bacterioplankton groups in China seas and global oceans. These bacterial groups play a special role in marine carbon cycling and light utilization but received little attention, including: *Cytophaga-Flavobacteria* (CF), which are proficient in degrading high molecular weight particulate organic carbon; planktonic *Archaea*, the metabolic potential and ecological functions of which are largely unknown; planktonic *Proteobacteria* with the ability of fixing CO_2; and mixtrophic aerobic anoxygenic photosynthetic bacteria (AAPB) and pigmented heterotrophic bacteria (PHB), which can facultatively utilize or absorb light.

Firstly, we chose the Yangtze River estuary (YRE) in the East China Sea (ECS) as a typical eutrophic water and systematically investigated the diversity pattern of CF, planktonic archaea, and CO_2 fixation proteobacteria there. CF cluster is one of the most abundant bacterial groups in the ocean. A newly designed CF specific 16S rRNA gene primer pair was used to construct two clone libraries from YRE and ECS bacterioplankton samples. An abundant CF diversity and a distinct diversity distribution pattern were revealed. Seventy partial 16S rRNA gene sequences of CF were clustered into 26 subgroups, including 7 subgroups that distributed only in the ECS, 17 subgroups that distributed only in the YRE, and 2 subgroups that were shared by both stations. In comparison, the CF species diversity was high in the ECS, but the CF subgroup diversity was high in the YRE. Cosmopolitan subgroups dominated both stations. Subgroups with the soil, coast, and freshwater origins were detected only in the YRE, in accordance with the geographical location and environmental features there. The adaptation of CF species to the two completely different stations with respect to hydrology and the development and succession of local CF populations resulted in these significant differences between the YRE and ECS CF communities.

By the construction of two archaeal 16S rRNA gene clone libraries from YRE bacterioplankton samples, we also revealed the presence of planktonic archaea in typical Chinese marine waters, which has been found to be cosmopolitan in marine ecosystems. Sequencing of the clones in 21 unique RFLP patterns showed the two archaeal groups: Marine Group I (MG I) that was affiliated with *Crenarchaeota* and Marine Group II (MG II) that was affiliated with *Euryarchaeota*. MG I dominated both libraries. A large part of sequences were most related to uncultured archaea, including 2 sequences that were in 98% similarity with a nitrification marine archeaon *Nitrosopumilus maritimus*, suggesting that archaea may play an important role in estuarine ecosystems.

Proteobacteria with the potential of fixing CO_2 is another understudied group in bacterioplanton. Here we for the first time successfully designed two primer pairs targeting the Form I and Form II *rbcL* genes (encoding RubisCO large subunit), respectively, and applied them to the inshore and offshore samples from the ECS. Our results showed that the Form I *rbcL* diversity was high at the inshore station with high salinity and low productivity, while the Form II *rbcL* diversity was high at the offshore station with low salinity and high productivity. A part of sequences showed a low similarity (60~78%) with their nearest neighbors in the GenBank database. The results indicate the presence of carbon-fixing proteobacteria in the ECS and suggest that we should draw more attention to their potential contribution in marine carbon cycling.

Secondly, we focused on a typical functional bacterial group in marine bacterioplankton – AAPB and systematically studied their diversity pattern in global oceans and co-evolution with environments. The diversity data of an AAPB marker gene, *pufM*, was collected from the surface seawater of the Pacific, Atlantic, and Indian oceans, and Chinese marginal seas as well. The results showed that AAPB populations were highly diverse in global oceans and, more importantly, their diversity decreased with the increased Chl.*a* concentrations. This finding indicated that AAPB diversity and abundance followed a reverse trend from oligotrophic to eutrophic oceans.

Depth profile of AAPB diversity was also investigated in three stations in the surface and upper twilight zones of the central Pacific, Atlantic, and Indian oceans by constructing six *pufM* gene clone libraries. The high AAPB diversity in oligotrophic oceans was further confirmed. Furthermore, we for the first time obtained abundant *pufM* sequences (136 sequences within 37 OTU) from deep twilight zones (200 m). Phylogenetic analysis showed that deep AAPB populations were diverse too, covering

all the subgroups that were found in surface stations. They had a lower diversity, GC and GC_3 contents but a higher dominance than surface stations. These results support the hypothesis that AAPB can utilize dim light to acquire energy and thus distribute into the layers blow euphotic zones.

To further probe the co-evolutionary relationship between anoxygenic photosynthetic bacteria and environment, we retrieved the *pufM* sequences and related source information of 89 anoxygenic photosynthetic bacterial cultures from public database. Phylogenetic analysis revealed that horizontal gene transfer (HGT) occurred in 11 of total 21 *pufM* phylogenetic subgroups. HGT occurred among species within not only the same class but also different phyla or subphyla. Source environmental features of anoxygenic photosynthetic bacteria were closely related to their phylogeny. Species from oxic and anoxic environments clustered into separate and distinct clades in the phylogenetic tree. HGT between ancient anoxygenic photosynthetic bacteria and long-term adaptive evolution into different niches may result in such coevolutionary events.

Then, in addition to the analysis of bacterial diversity by culture-independent molecular methods, we also employed culture-dependent methods to study the diversity, abundance, and light absorption features of pigmented heterotrophic bacteria (PHB), which is another interesting bacteria group with respect to light utilization. PHB cultures were isolated from the coast and shelf of China seas and adjacent Pacific and were identified by 16S rRNA gene analysis. The results showed that PHB abundance and their percentage in total cultivable bacterial amount decreased from coast to open ocean with the highest values (9.9×10^3 cells mL^{-1} and 39.6%, respectively) occurring at the YRE station. In the depth profiles, PHB were only distributed in the euphotic zones and the highest values of both abundance and percentage proportion occurred at the surface stations. Total 247 PHB isolates were screened with 16S rRNA gene PCR-RFLP and then sequenced. The PHB strains we obtained covered 6 major bacterial groups (*α-Proteobacteria*, *γ-Proteobacteria*, *Actinobacteria*, *Bacilli*, *Flavobacteria*, and *Sphingobacteria*) and 25 genera. Dominant group differed among different stations, and different genus showed different colors, including golden yellow, yellow, red, pink, and orange etc. Cellular pigments absorbed the light between 450~550 nm. These results indicated that PHB were widely distributed in marine environments with high diversity. They may play a unique and important role in marine ecosystems due to the absorption

and utilization of light.

Finally, we established a set of new criterion for clustering *pufM* gene sequence by distance methods and a new method for assessing 16S rRNA gene copy number in bacterial genomes by combining PFGE and PCR amplification. The cutoff for discriminating species, genus, and (sub-)phylum based on *pufM* gene sequences were 0.06, 0.15, and 0.48, respectively.

Keywords: Marine Bacterioplankton; *Cytophaga-Flavobacteria*; Planktonic Archaea; Carbon-fixation *Proteobacteria*; Aerobic Anoxygenic Photosynthetic Bacteria (AAPB); Pigmented Heterotrophic Bacteria; 16S rRNA gene; *pufM*; *rbcL*; Phylogenetic analysis; Genetic Diversity; China Seas; Open Oceans; Twilight Zones; Environmental Adatation Mechanisms; Co-evolution; PFGE

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